Regional distribution and ultrastructural characteristics of growth hormone - secreting (GH) cells in the Adenohypophysis of Tokara goat.

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Regional distribution and ultrastructural characteristics of growth hormone - secreting (GH) cells in the *Adenohypophysis* of Tokara goat.

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The adenohypophysial GH cells in Tokara goats were examined by light and electron microscopic immunocytochemistry. The immunoreactive GH cells distributed throughout the *Pars distalis* except for *Zona tuberalis* (ZT) and were recognized on a half of all secretory cells in the region in both sexes. The percentage area of GH immunopositive granules were 19.2% in male and 22.7% in female in that region. GH cell had a spherical, oval or irregular shaped nucleus, round or rod-like mitochondria, a lot of secretory granules, jaxtanuclear Golgi apparatus and rough endoplasmic reticulum running among granules. The secretory granules varied in size from cell to cell. Their average diameters were 329±15 nm in male and 350± 15 nm in female. More than 90% of GH cells had the granules of 250 to 400 nm in the mean diameter in both sexes. Sex differences in the morphological features of GH cells were not recognized. By the comparison of the granule size with those of the other reports, it was clarified that Tokara goats showed rather small size in domestic ruminants.

INTRODUCTION

Tokara goat found as a semiwild breed in Tokara islands south of Kyushu in Japan, can be expected to be an experimental animal in the special researches of farm ruminants at a lower cost and a great convenience. This small ruminant (body weight: 30.4 kg in male, 19.3 kg in female at 35 months old) is reproduced and reared easily under general management (Manda, 1986). However, fundamental observation of the hypophysis is remained to be investigated morphologically.

In the *Adenohypophysis* of ruminants and other several animal species, it is known that *Zona tuberalis* (ZT) composed of basophils mainly situates from cranioventral to central region of the *Pars distalis*, and that acidophils such as GH cells or prolactin cells don't distribute almost throughout ZT (Dawson, 1937; El Etreby and Fath El Bab, 1978; Hanström, 1966; Heath, 1970; Mikami and Daimon, 1968; Nishimura *et al.*, 1990).

Using the immunocytochemical method, GH cells in the *Adenohypophysis* have been characterized and distinguished from the other secreting cells in the light or electron microscopic investigations in many animals. Although GH cells have numerous, spherical secretory granules, the granule size is variable among the species. In ruminants, the different granule sizes were reported among the species, and also in the same species by different authors (Dacheux and Dubois, 1976; Fumagalli and Zanini, 1985; Gómez *et al.*, 1989; Heath, 1970; Mikami, 1970; Sánchez *et al.*, 1994; Shirasawa *et al.*, 1985; Thorpe *et*

al., 1990; Yamaguchi *et al.*, 1988).

In the present study, the *Adenohypophysis* of Tokara goat was observed under light and electron microscopes after treating with the immunocytochemical method for detecting GH cells. Distribution and ultrastructure of the GH cells were shown and discussed by comparison with results of the other species of ruminants.

MATERIALS AND METHODS

Materials: Three male and 4 female (anoestrus) adult Tokara goats (12–16 months old) breeded in Kyushu University Farm were used. Their body weights were 27.1±2.6 kg in male and 20.1±3.9 kg in female. They were killed by bleeding under deep anesthesia with sodium pentobarbitone between 10:00 to 11:00 a.m.. Pituitary gland was excised and divided into two blocks at mid-sagittal plane. The right block was destined for light microscopic observation and the left for electron. Besides, the nomenclatures were in accordance with the Nomina Anatomica Veterinaria Japonica (3rd ed.).

Procedures for light microscopic observations: The right block of the hypophysis was fixed with a sublimate-formalin, a solution of saturated mercury chloride and formalin (9:1), for 24 hours at room temperature. After being washed by running water and dehydrated through ethanol series, the tissue was embedded in paraplast and cut into sagittal sections at 4μm thick. For detecting GH cells, the sections were stained by avidin-biotin-peroxidase complex (ABC) method with Vectastain ABC kit (PK–4001D, Vector Laboratories inc., USA) according to Hsu *et al.* (1981). The primary antiserum in this method was rabbit anti-ovine GH serum (1:2000, UCB-Bioproducts S.A.). Counterstaining by hematoxylin was also done.

The controls of this method were performed as follows: 1) replacement of primary antiserum by PBS, 2) preabsorption of primary antiserum by corresponding antigen, 3) omission of biotinylated anti-rabbit IgG or avidin-biotinylated-peroxidase complex solution in ABC procedure. No specific reactions were observed in these controls.

Procedures for electron microscopic observations: The small tissues taken from mediocaudal region of the left block were prefixed by 1% paraformaldehyde–3% glutaraldehyde in phosphate buffer (pH 7.4) at 4 °C overnight and postfixed by buffered 1% osmium-tetraoxide solution at 4 °C for 2 hours. After being dehydrated in ethanol series and replaced by propylene oxide, the tissue was embedded in quetol 812 (Nissin EM co. ltd., Tokyo), cut into ultrathin sections by use of ultra-microtome MT–1 (Dupont Instruments, USA), and mounted on nickel grids.

The sections were stained with protein A-gold method for detection of growth hormone (Bendayan and Zollinger, 1983). The primary antiserum was the same as above. Protein A-gold complex (gold particle: 10 nm) was obtained from Amersham International plc. (UK). After counterstaining with uranyl acetate and lead acetate, the specimen was observed under electron microscope (H-600, Hitachi, Japan).

Morphometric analysis : Percentage distribution of GH cells was observed in the 3 regions of *Pars distalis*, namely mediodorsal over ZT, mediocaudal, and lateral regions (Fig. 1).

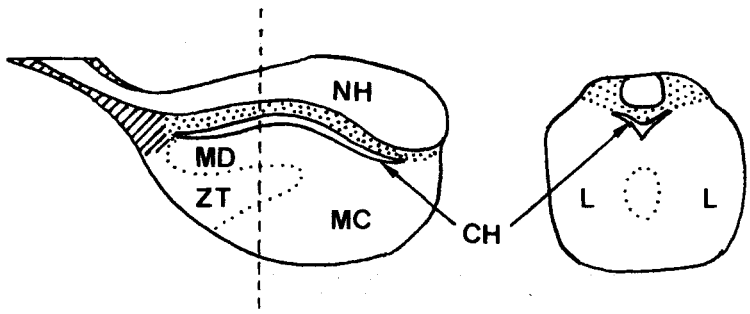


Fig.1. Schematic diagram of Tokara goat Hypophysis. The left picture indicates mid- sagittal plane of Hypophysis and right one indicates frontal section that corresponds the dotted line of the left. Dots indicate *Pars intermedia*. Oblique lines indicate *Pars tuberalis*. CH: *Cavum hypophysis*, L: Lateral region of *Pars distalis*, MC: Mediocaudal region of *Pars distalis*, MD: Mediodorsal region over *Zona tuberalis*, NH: *Neurohypophysis*, ZT: *Zona tuberalis*.

Every two light micrographs (×200) in each region in three preparates were taken at random and the numbers of all secretory cells and GH cells were counted. Moreover, percentage area of GH immunopositive granules in each region was analyzed by image analysis system (Nexus Qube, Nexus inc., Tokyo).

On electron micrographs (×15000 or ×20000), more than 50 immunoreactive GH cells in each animals were observed. The diameters of 15–100 secretory granules (maximum at long axis) in each cell were measured and their means were determined.

Mean and standard deviation were used to analyze statistical difference at Student's t-test.

RESULTS

Light microscopic observations

By ABC method, immunopositive GH cells distributed only in the *Pars distalis* except for ZT of all regions of the hypophysis. GH cells showing different staining intensities from cell to cell were middle or small in size and oval, cylindrical or polygonal in shape (Fig. 2). Sex difference of morphological features in GH cells was not recognized.

About a half number of secretory cells in the *Pars distalis* except for ZT were GH immunopositive (Table 1). In the whole area examined, the percentage distribution of GH cells was 49.3±3.1% in male and 49.3±4.6% in female. On the other hand, the smallest percentage distribution of GH cells in each region was shown in the mediocaudal region in male (44.6±7.0%) and the mediodorsal over ZT in female (41.8±8.5%). Then, regional difference of the GH cell percentage was significant between the mediodorsal and mediocaudal region only in female ($p<0.05$). The percentage in the mediodorsal region was significantly larger in male than in female ($p<0.05$).

The percentage area of GH immunopositive granules in the *Pars distalis* was shown

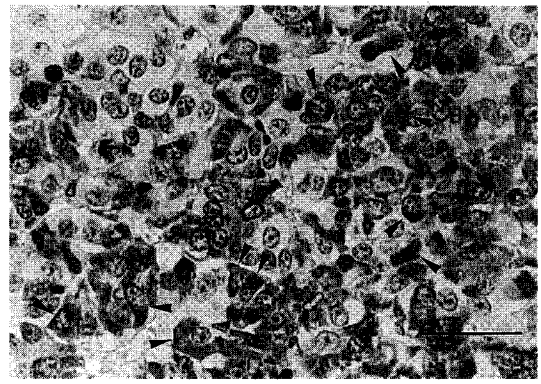


Fig.2. Light micrograph of GH cells of male Tokara goat. Arrowheads indicate GH cells. Bar indicates 30μm. $\times$400

Table 1. Percentage distribution of GH cells in male and female Tokara goat *Adenohypophysis*.

	No. of goats	Mediodorsal region over *Zona tuberalis*	Mediocaudal region	Lateral region	Total
Male	3	52.9 $\pm$ 2.19^{ax}	44.6 $\pm$ 6.98^{ax}	52.0 $\pm$ 4.29^{ax}	49.3 $\pm$ 3.12^{x}
Female	4	41.8 $\pm$ 8.52^{ay}	53.7 $\pm$ 1.07^{bx}	52.5 $\pm$ 4.59^{abx}	49.3 $\pm$ 4.55^{x}

Mean$\pm$ standard deviation.

^{a,b} : Means with same superscript do not significantly differ between regions ($p<0.05$).

^{x,y} : Means with same superscript do not significantly differ between sexes ($p<0.05$).

in table 2. In the whole area examined, the percentage area was 19.2% in male and 22.7% in female. The percentage area did not show significant regional differences in both sexes and also sex difference in each region.

Electron microscopic observations

The gold particles produced by immunoreaction to the GH antiserum were localized only on the secretory granules (Figs. 3–6). On the electron micrographs of the GH cells, the dense secretory granules exhibited spherical or oval profiles with smooth surface (Figs. 3–6). They were numerous and distributed evenly among rough endoplasmic reticulum of one or a few cisternae. Spherical or oval mitochondria with a few cristae were also observed (Fig. 4). Jaxtanuclear Golgi apparatus showed well developed cisternae that distended into vacuoles at their periphery (Fig. 5). The nucleus situated eccentrically was usually spherical or oval in shape and sometimes showed the irregular contours with indentation of the nuclear envelope (Figs 3 and 4). The proportions of the cells whose nuclei had smooth contours were 67.5$\pm$25.5% in male and 69.6$\pm$19.6% in female. The great proportion of the nucleus was occupied by euchromatin of low electron density and prominent nucleoli were also observed (Fig. 4).

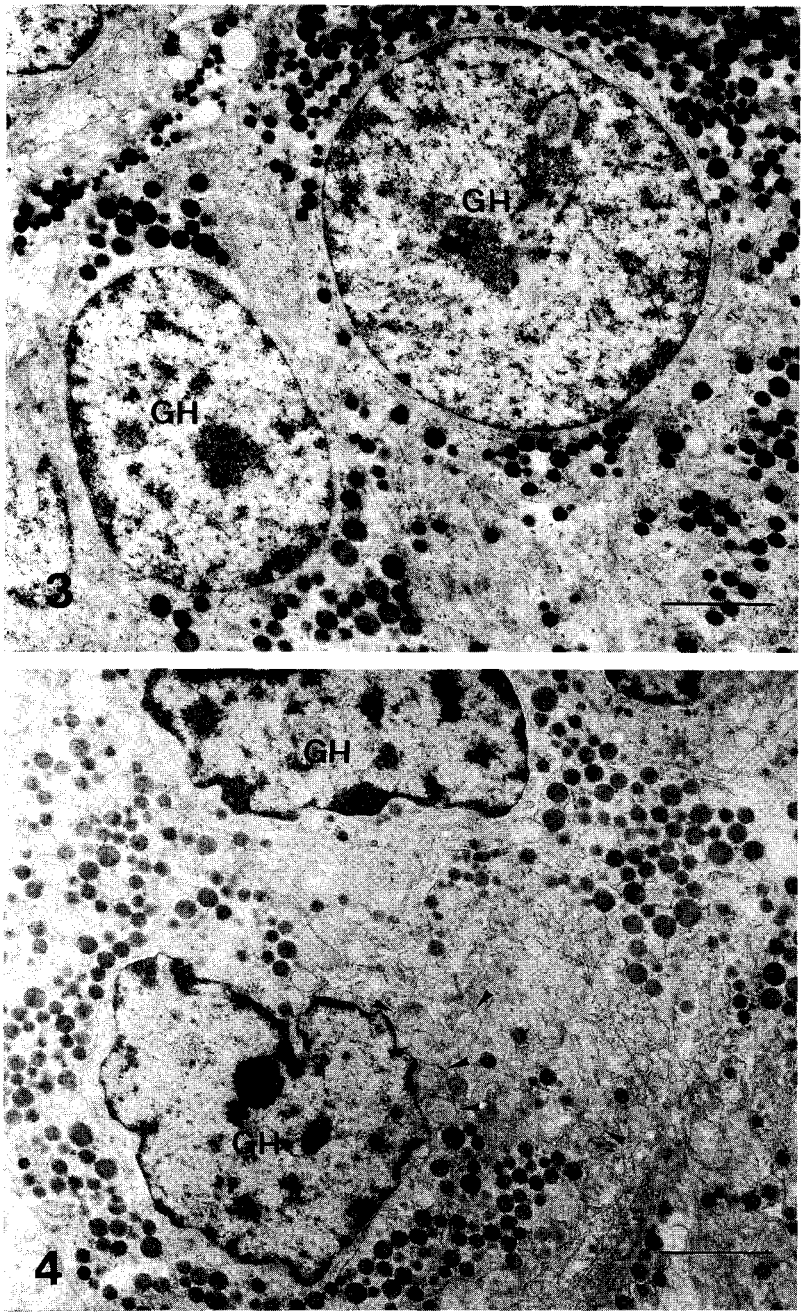
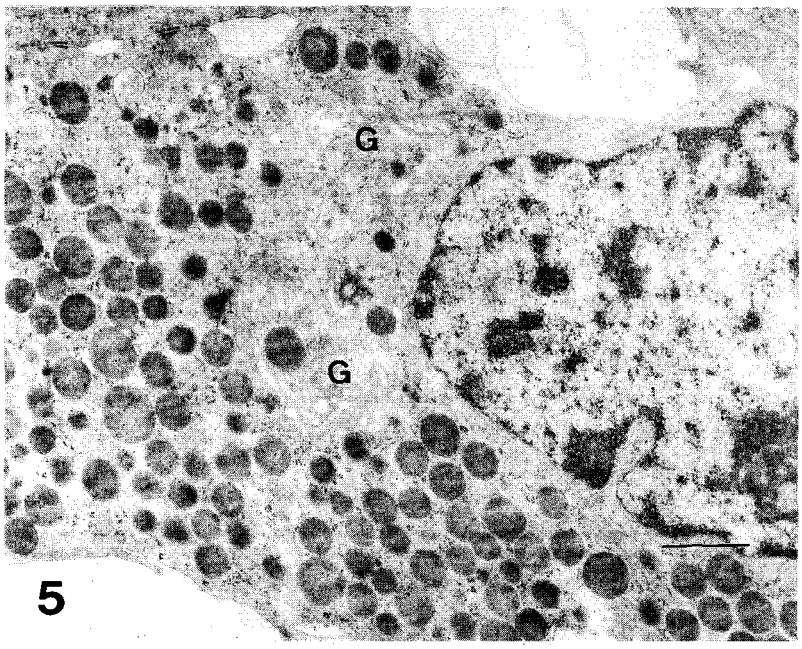


Fig.3. Electron micrograph of GH cell of male Tokara goat. These GH cells have spherical or oval nuclei. Bar indicates 2μm.

Fig.4. Electron micrograph of GH cell. These GH cells have irregular shaped nuclei with indentation on their surface. Arrowheads indicate mitochondria. Bar indicates 2μm.



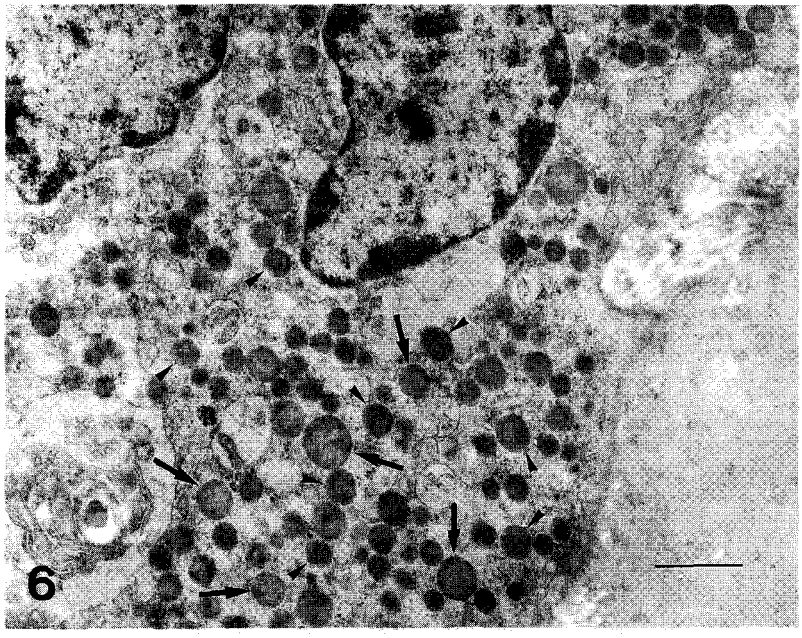


Fig.5. Electron micrograph of GH cell of female Tokara goat. A few Golgi apparatus (G) is observed in the jaxtanuclear position. Bar indicates 1 μm.

Fig.6. Electron micrograph of GH cell of female Tokara goat. This GH cell has immunopositive granules (arrowheads) and immunonegative granules (arrows) simultaneously. Bar indicates 1 μm.

In most of GH cells, all of the secretory granules were immunopositive, but several cells contained both of the immunopositive and immunonegative granules simultaneously, in which the former was usually smaller than the latter (Fig. 6).

The secretory granules of GH cells covered wide range in their size in all animals examined. When the average diameter was determined in each cell, the averages of the values were not different among individuals. The average diameters were 329±15 nm in male (n=3) and 350±15 nm in female (n=4). The histograms in figure 7 indicate the frequency of GH cells in each class of the average size of secretory granules in the cell. More than 90% of the GH cells contained the granules of 250–400 nm in the mean diameter and the maximum class of frequency was 300–350 nm in both sexes.

DISCUSSION

As Manda (1986) reported that the first delivery time of Tokara goat is 451.4 days old and pregnant period is 147.5 days, the animals in this study (12–16 months old) are seemed to mature sexually. After the age, the goats show yet the steady growth (Manda, 1986). Therefore, it was suspected that they did not complete the growth in the epiphysial cartilage of bone that is known as one of the target organs of growth hormone, and that the GH cells were still active.

Concerning about the light microscopic observations, GH cells of Tokara goat in this study were similar to those of kids reported by Gómez *et al.* (1989) in the shape and distribution. On the other hand, there were few reports that were examined about the distribution of GH cells in each region in the *Pars distalis* of the *Adenohypophysis*. In this study, the percentage distribution of GH cells was larger in the mediocaudal than in the mediodorsal region in female and in female than in male in the mediocaudal region (Table 1). However, the percentage area of GH immunopositive granules didn't show any regional and sex differences (Table 2). These results indicated that the granule volume in the region did not always related to the cell number and that GH cell could contain the granules of different size and number under various physiological conditions. It was not apparent that GH cells with both the immunopositive and immunonegative granules play a main role to decrease the granule volume relative to the cell number.

Gómez *et al.* (1989) reported that the percentage of GH cells in kids was 34 % of the secretory cells in the all adenohypophysial area. In this study, a half number of the secretory cells in *Pars distalis* except for ZT of Tokara goat were GH cells. It was suspected that these differences were brought from the different counting methods about the secretory cells in ZT, ages or breeds.

Table 2. Percentage area of GH immunopositive granules in the pars distalis of Tokara goat *Adenohypophysis*.

	No. of goats	Mediodorsal region over *Zona tuberalis*	Mediocaudal region	Lateral region	Total
Male	3	18.8 ± 2.30	15.0 ± 4.71	23.8 ± 3.50	19.2 ± 4.42
Female	4	20.3 ± 3.53	20.4 ± 3.28	27.3 ± 8.29	22.7 ± 4.02

Mean± standard deviation.

Although spherical or oval nuclei usually appeared in GH cells, the nuclei were sometimes irregular in shape by the indentation of the nuclear envelope. As this irregular nuclei increase the surface area of their envelope and meet the greater functional demands, the GH cells having this type nucleus may be very active in the hormone secretion. Accordingly, in the GH cell with an irregular nucleus, prominent nucleolus, well developed rough endoplasmic reticulum and much mitochondria were also observed, indicating active production of the hormone.

One of the criteria to distinguish and characterize a cell type from the other in the *Adenohypophysis* is size and shape of secretory granule. In GH cells, spherical or oval granules have been reported to be 343 nm in male goat (Shirasawa *et al.*, 1985) or 391 nm in male and 427 nm in female kids (Gómez *et al.*, 1989). On the other hand, in female goats under different physiological conditions, the diameter of GH granules are 569 nm

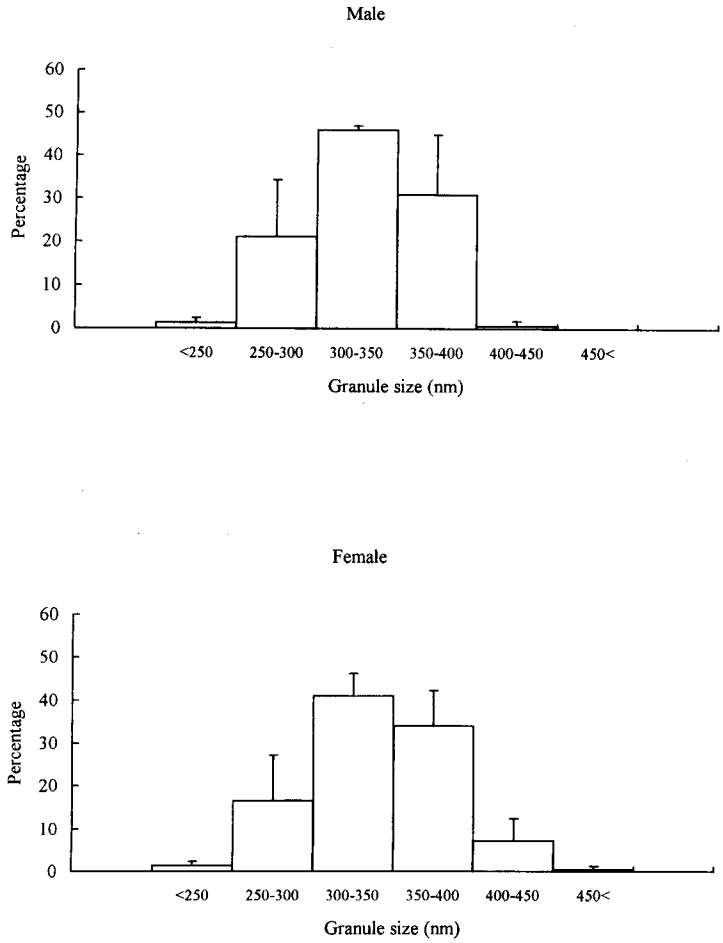


Fig.7. Distribution of the mean diameter of GH secretory granules in each cell in male and female Tokara goat.

during anoestrum, 543 nm during pregnancy and 611 nm during lactation (Sánchez *et al.*, 1994). In this study, Tokara goat showed the granule diameters of 329 nm in male and 339 nm in female. The granules of 250–400 nm in the mean diameter were observed at more than 90% of GH cells, where the maximum class of frequency was 300–350 nm in both sexes. The results in this study coincide with those by Shirasawa *et al.* (1985) in male but are different from those of Gómez *et al.* (1989) and Sánchez *et al.* (1994). From these results in goat, it was suggested that the granule size of GH cells could vary with the breed, sex and physiological condition.

As the granule sizes vary more widely in bovine by the authors such as 400–450 nm by Mikami (1970), 350–500 nm by Heath (1970), 500–900 nm by Dacheux and Dubois (1976), and 200–900 nm by Fumagalli and Zanini (1985) and Yamaguchi *et al.*(1988), the previous difference in goat is rather small. In sheep, the mean granule size is 300 nm (Thorpe *et al.*, 1990). These results in the other ruminants indicated that in the granule size the Tokara goats were rather similar to the sheep than the other goat breeds and was smaller than bovine.

Somatomammotroph has been reported as a cell to contain two kinds of hormones, growth hormone and prolactin in cow (Fumagalli and Zanini, 1985), sheep (Thorpe *et al.*, 1990) and goat (Sánchez *et al.*, 1994). In this study, some GH cells also had two kinds of granules, smaller GH immunopositive and larger immunonegative. It was seemed that these cells might be somatomammotrophs from the characteristics of the granules.

In conclusion, the *Adenohypophysis* of Tokara goat was characterized by the results as follows: 1) GH cells distributed throughout the *Pars distalis* except for ZT and they were observed in a half number of secretory cells in the region. 2) GH cells had many spherical or oval secretory granules and their average diameters among the GH cells were 329 ± 15 nm in male and 350 ± 15 nm in female. Moreover, more than 90% of the GH cells contained the granules of 250–400 nm in the mean diameter. 3) Sex differences were not observed in percentage area of the GH granules and ultrastructure of GH cells.

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